

Serial No.: 10/761,717  
Attorney Docket No.: 291-0002US  
Response to Restriction

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

Claim 1 (original): A method of producing insulinotropic GLP-1(7-36) polypeptide and/or GLP-1 analogs comprising:

- (a) introducing two individual restriction endonuclease cleavage sites capable of forming a hybrid site to two terminals of a gene which may encode the GLP-1(7-36) polypeptide or GLP-1 analogs;
- (b) ligating cohesive ends to form a hybrid site after digestion with restriction endonucleases, and cloning into a vector N copies of a resulting series-linked GLP-1(7-36) gene, GLP-1 analog gene, or combination genes encoding GLP-1(7-36) polypeptide or GLP-1 analogs, wherein N is an integer from 1 to 32;
- (c) transforming a vector containing the series-linked gene into a host cell;
- (d) expressing into the host cell a fusion protein comprising N copies of the series-linked GLP-1(7-36) polypeptide, GLP-1 analog or combination thereof, but without any carrier protein;
- (e) cleaving the fusion protein; and
- (f) separating and purifying the GLP-1 (7-36) polypeptides and/or GLP-1 analogs.

Claim 2 (original): The method according to claim 1 wherein the two restriction endonucleases capable of forming a hybrid site are Bgl II and BamH I.

Claim 3 (original): The method according to claim 1 wherein the two restriction endonucleases capable of forming a hybrid site are Sal I and XhoI I.

Claim 4 (original): The method according to claim 1 in which said vector contains N copies of the series-linked gene, wherein N is an integer from 2 to 32.

Claim 5 (original): The method according to claim 4 in which the said vector contains N copies of the series-linked gene, wherein N is an integer from 8 to 32.

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Claim 6 (original): The method according to claim 5 in which the said vector contains N copies of the series-linked gene, wherein N is 16.

Claim 7 (original): The method according to claim 5 in which the said vector contains N copies of the series-linked gene, wherein N is 32.

Claim 8 (original): The method according to claim 6 wherein said vector is the one contained in the deposit of CGMCC Accession No.0599.

Claim 9 (original): The method according to claim 1 in which said host cell may express a fusion protein containing N copies of a polypeptide, wherein N is an integer from 1 to 32.

Claim 10 (original): The method according to claim 9 in which said host cell may express a fusion protein containing N copies of a polypeptide, wherein N is an integer from 8 to 32.

Claim 11 (original): The method according to claim 10 in which said host cell can express a fusion protein containing N copies of a polypeptide, wherein N is 16.

Claim 12 (original): The method according to claim 10 in which said host cell can express a fusion protein containing N copies of a polypeptide, wherein N is 32.

Claim 13 (original): The method according to claim 9 wherein said host cell is a prokaryotic cell.

Claim 14 (original): The method according to claim 13 wherein said host cell is *Escherichia coli* JM103, JM109, HB101, or DH5 $\alpha$  or C600.

Claim 15 (original): The method according to claim 14 wherein said host cell is the one contained in CGMCC Deposit No. 0599.

Claim 16 (original): The method according to claim 1 wherein said protease used to cleave the fusion protein is Clostrispan or Trypsin.

Claim 17 (original): The GLP-1 (7-36) polypeptide and/or GLP-1 analog produced according to the method of claim 1.

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Claim 18 (original): The GLP-1 (7-36) polypeptide according to claim 17, having an amino acid sequence of which is shown in Formula I:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-  
Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-OH

Formula I

Claim 19 (original): A method of producing an expression vector comprising multiple tandem copies of a gene encoding a desired polypeptide comprising the steps of:

(a) constructing a vector comprising the gene and four individual restriction enzyme sites A - D in a relative order A - C - gene - B - D, wherein restriction enzyme sites C and B are capable of forming a hybrid site lacking restriction enzyme sites C and B;

(b) digesting an aliquot of the vector comprising the gene with endonucleases C and D and isolating a resulting double digested gene fragment;

(c) digesting a second aliquot of the vector with endonucleases B and D and isolating a resulting double digested vector including the gene;

(d) ligating the double digested gene fragment and the double digested vector comprising the gene to form a vector comprising N tandem copies of the gene linked by the hybrid site lacking restriction enzyme sites C and B; and

(e) repeating steps b - d, wherein each repeating series of steps begins with the vector product of step d such that the N tandem copies of the gene double with each series.

Claim 20 (original): The method of claim 19, wherein the gene encodes one or more additional N-terminal amino acids selected from the group consisting of: Met; Arg; Met-Arg; Met-Met-Arg; Asp-Asp-Asp-Asp-Lys; and combinations thereof.

Claim 21 (original): The method of claim 19, wherein the polypeptide is insulinotropic.

Claim 22 (original): The method of claim 21, wherein the insulinotropic polypeptide selected from the group consisting of: GLP-1(7-36); GLP-1 analogs; and exendin-4 analogs.

Claim 23 (original): The method of claim 22, wherein N is an integer from 2 to 32.

Claim 24 (original): The method according to claim 23, wherein N is an integer from 8 to 32.

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Claim 25 (original): The method of claim 19, wherein restriction endonucleases sites C and B capable of forming a hybrid site are Bgl II and BamH I.

Claim 26 (original): The method of claim 19, wherein restriction endonucleases sites C and B capable of forming a hybrid site are Sal I and XhoI I.

Claim 27 (original): A method of producing an insulinotropic polypeptide comprising:  
(a) expressing into a host cell a fusion protein comprising 2 - 32 tandem copies of the insulinotropic polypeptide, wherein each copy comprises a cleavable N-terminal Arg or cleavable spacer;  
(b) isolating the fusion protein from the host cells;  
(c) cleaving the fusion protein at the cleavable N-terminal Arg or cleavable spacer; and  
(d) separating and purifying the insulinotropic polypeptide.

Claim 28 (original): The method of claim 27, wherein the fusion protein is cleaved by treatment with a compound selected from the group consisting of: cyanogen bromide, alkaline proteases, enterokinases, endopeptidases, and combinations thereof.

Claim 29 (original): The method of claim 28, wherein the alkaline protease is trypsin and internal lysine groups are acetylated prior to trypsin treatment.

Claim 30 (original): The method of claim 29, wherein the internal lysine groups are acetylated by treatment with an anhydride followed by deprotection after trypsin treatment.

Claim 31 (original): The method of claim 30, wherein the anhydride is selected from the group consisting of: acetic anhydride; maleic anhydride; citraconic anhydride, and 3, 4, 5, 6-tetrahydrophthalic anhydride.

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Claim 32 (original): The method of claim 27, wherein the insulinotropic polypeptide is selected from the group consisting of: GLP-1(7-36) (SEQ ID NO:1), GLP-1(7-36)-NH<sub>2</sub> (SEQ ID NO:2), Gly<sup>8</sup>-GLP-1(7-36) (SEQ ID NO:4), Val<sup>8</sup>-GLP-1(7-36) (SEQ ID NO:5), Asp<sup>11</sup>-GLP-1(7-36) (SEQ ID NO:6), Ala<sup>16</sup>-GLP-1(7-36) (SEQ ID NO:7), Glu<sup>22</sup>-GLP-1(7-36) (SEQ ID NO:8), His<sup>23</sup>-GLP-1(7-36) (SEQ ID NO:9), Glu<sup>24</sup>-GLP-1(7-36) (SEQ ID NO:10), Trp<sup>26</sup>-GLP-1(7-36) (SEQ ID NO:11), Ala<sup>27</sup>-GLP-1(7-36) (SEQ ID NO:12), Glu<sup>30</sup>-GLP-1(7-36) (SEQ ID NO:13), Asp<sup>33</sup>-GLP-1(7-36) (SEQ ID NO:14), Glu<sup>34</sup>-GLP-1(7-36) (SEQ ID NO:15), Thr<sup>35</sup>-GLP-1(7-36) (SEQ ID NO:16), Gly<sup>8</sup>-Glu<sup>24</sup>-GLP-1(7-36) (SEQ ID NO:17), Leu<sup>8</sup>-Ala<sup>33</sup>-GLP-1(7-36) (SEQ ID NO:18), and exendin-4 analogs.

Claim 33 (original): The method of claim 32, wherein the insulinotropic polypeptide is GLP-1(7-36) (SEQ ID NO 1) and the cleavage spacer is an N-terminal Arg.

Claim 34 (original):The method of claim 32, where the insulinotropic polypeptide is GLP-1 (7-36) (SEQ ID NO 1) and each GLP-1 copy is preceded by an N-terminal Met-Arg.

Claim 35 (original): The method of claim 34, wherein the isolated fusion protein is treated with cyanogen bromide followed by cleavage with clostripain protease.

Claim 36 (original): The method of claim 27, wherein the fusion protein has a coding sequence selected from the group consisting of: SEQ ID NO: 29 and SEQ ID NO:30.